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(PLATES 16, 17, 18).

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Development of the gametophytes of *Podocarpus nivalis* up to fertilization has been described in a recent paper (Boyle and Doyle, 1953) and comparison made with corresponding phases in other podocarps. This paper follows the embryogeny, including polyembryony, up to the organization of the multicellular clumps destined to form the embryo proper. Comparison is also made with other eupodocarps.

A. EARLIER WORK.

Accounts of embryogeny in species of *Eupodocarpus* have been given by Coker (1902), Stiles (1912), Sinnott (1913), Tahara (1941), Buchholz (1941), and Brownlie (1953). These accounts are in many features discordant, and none is complete.

Coker (1902) gave the first account of development in any podocarp. As already referred to in the first paper of this series it is uncertain which species he used. Called by him *P. coriaceus* it was more probably (Buchholz, 1941) *P. macrophyllus* var. *maki*. It was however some species of the *Eupodocarpus* section. Missing the first sporophytic division of the fusion nucleus his account begins with two nuclei at the base of the archegonium, the location of the first division being thus unknown. Further nuclear divisions produce 16 free nuclei followed by the stage of a walled, tiered proembryo. This showed a single terminal binucleate embryo cell, a second or middle suspensor tier of 14, and an upper tier of 14 open to the archegonium above, and called by Coker the ' rosette ' tier. The record of a binucleate embryo cell is thus an early one, but he gave no clue either to its origin or to the manner of organization of the whole mature proembryo except to suggest that the ' rosette ' tier had possibly been formed by division from the second tier. If this were so then at *first* wall formation there would have been a basal single embryo cell and an open tier of 14 which gave by division the upper ' rosette ' and the middle suspensor tiers. He further suggested that the single

embryo cell first divided by two successive longitudinal walls. For this however there is no evidence given except (*a*) his Fig. 40 which actually shows something quite different and (*b*) the apparent splitting of the embryo into four. Some splitting or cleavage may have taken place, but Coker's suggestion, unsupported by evidence, is definitely open to another simpler explanation which will be described later under polyembryony. The nature of the nuclear change in the embryo cells from the early binucleate condition to the later uninucleate condition was not considered. This account is obviously incomplete.

Stiles (1912) gave only a short account of his material which was the true *P. macrophyllus*. He states that the fusion nucleus passes to the archegonium base and there divides, but almost certainly this was not seen and only inferred following Coker. He also speaks of 16 free nuclei being formed, but did not see the early walled proembryo, and later saw no case of splitting.

Sinnott (1913), in his long article on podocarps, gives records for the three related New Zealand forms—*P. totara*, its var. *Hallii*, and *P. nivalis*. Claiming that development in all three is essentially similar he gives a general account to cover the group. This account differs radically from that of Coker and Stiles. According to Sinnott the first division occurs in the position of the fusion nucleus, the two nuclei produced passing to the base. Subsequent mitoses give 16 free nuclei which are organised at wall formation into a proembryo of three tiers—a single binucleate embryo cell, a suspensor tier of 7—10, and a ' rosette ' tier above of " a few cells which soon go to pieces ". This again differs from Coker's account not only in the smaller number of suspensor cells but in the origin of the so-called ' rosette ' tier, which, according to Sinnott, is formed round some of the original nuclei and not derived by division from a second tier. He speaks too of ' rosette ' *cells* leaving it doubtful if these are closed above or open to the archegonium as in Coker's material. He says too that the single embryo cell first divides by a longitudinal wall and then by transverse ones, but no evidence is given, nor is the change from the binucleate to the uninucleate condition of embryo cells considered. Instances of " budding " are stated to have been " only rarely observed " but no detail whatsoever is given about this aspect.

In this unsatisfactory position, with nothing available save the two almost completely discordant accounts of Coker and Sinnott, the question remained for nearly 30 years till Tahara (1941) gave another short account of development in *P. macrophyllus*.

Tahara records, but without accompanying convincing illustration or data, *two* mitoses in the position of the fusion nucleus the resulting *four* nuclei passing to the base, thus introducing still another discordant feature. Further divisions produce 16 free nuclei, the only point in which all agree. The subsequent development is said to vary. From the 16-nucleate stage a walled proembryo is produced which may show two *or* three tiers. The basal tier again consists of a single binucleate cell, in one instance only were two such cells seen in this tier. The cells of the second tier are said to vary from 8 to 14. If 14 nuclei go to the

primary formation of this tier the cells so formed may close above, and in such cases no third or ' rosette ' tier is formed. But in other cases division in this tier may give, as suggested by Coker, an upper tier and a suspensor tier proper. When less than 14 nuclei go to make the primary second tier an upper ' rosette ' is formed, at the same time as the other tiers, from others of the primary nuclei without further division. Thus, apparently, something like the two different behaviours described respectively by Coker and Sinnott from their material could both occur in the proembryo formation of this material of *P. macrophyllus*. Such of course may occur, but the evidence given is very unconvincing and will be discussed later after the details have been described for *P. nivalis*. No embryo cleavage was recorded nor again was consideration given to the change from the binucleate to the uninucleate condition of the embryo cell.

Brownlie (1953) has recently investigated four eupodocarps native to New Zealand, viz., *P. totara*, its var. *Hallii*, and *P. nivalis*, already investigated by Sinnott, and also *P. acutifolius*. He states that as all are essentially similar in development one general account suffices for the group. For the early embryogeny up to the fully formed proembryo he repeats the type of development given by Sinnott for the same group. It is uncertain from the text whether he personally followed these stages or whether, as a preliminary to his own work at later stages, he has simply taken Sinnott's account. He gives no figures, referring for such to Sinnott. As Sinnott's account, at least of *P. nivalis*, is inaccurate, incomplete, and misleading, based obviously on insufficient material, it must be assumed that Brownlie, apparently not aware of Tahara's (1941) paper or of the papers by Looby and Doyle (1944 *a*, *b*) on *P. andinus*, simply accepted Sinnott's records without personal investigation of these stages. In the embryogeny, as described by Sinnott, the 16 primary nuclei are said to organize three tiers—one embryo cell, 7-9 suspensors, and an indefinite ' rosette ' tier above. Aware from Buchholz's work (1941) that other eupodocarps may have more than one embryo cell and up to 14 suspensors Brownlie also comments on the obvious fact that if, to the total in such cases, some ' relict ' upper ' rosette ' nuclei are added, then, the number of original free nuclei would appear to have been in excess of the 16 characteristic of his investigated New Zealand types. As the sequel will show all such apparent confusions and discrepancies simply arise from a lack of appreciation of the nature of podocarpean development and of the possible range of variation in proembryo cell numbers even within one individual.

He records two embryo-cells as an " occasional " occurrence and has some comments on cleavage polyembryony which will be discussed later.

Buchholz, in his many contributions to conifer embryogeny, was essentially interested in post-proembryo stages and in the nature of the cleavage polyembryony present, if any, so that in most cases only a general idea of the actual proembryo condition can be got from those forms investigated by him, and that mainly by inference. Nevertheless some information of interest is available. Thus in his account of later development in seven eupodocarps he records that there may quite

commonly be as many as 3 terminal binucleate cells, and that the suspensor number averages 12 in most species in contrast to the average of 8 recorded by Sinnott for the New Zealand types (Buchholz 1941). In this paper too he was the first to approach the necessity of following the change from the binucleate condition of the primary embryo cells to the uninucleate condition of later normal development, and put forward suggestions based on appearances in *P. totara*, suggestions later confirmed and extended by Looby and Doyle (1944 *b*) for *P. andinus*. A return will be made however to all these points as well as to his ideas on cleavage polyembryony.

It is clear from this survey that it was necessary to follow in reasonable detail the immediate post-fertilization stages in at least one eupodocarp. This has been done for *P. nivalis*.

B. Embryogeny in *P. nivalis*.

This account follows on directly from the last stage recorded in the earlier paper (Boyle and Doyle 1953) in which Fig. 29 showed fertilization stages completed and the organization of the diploid fusion nucleus in the upper half of the archegonium.

(*a*) *Proembryo, free nuclear stage.*

The fusion nucleus, still in the upper denser cytoplasm, undergoes the first sporophytic division more or less *in situ*, the mitosis (Pl. 16, 1), which is intranuclear, largely filling the nuclear area. The two resulting nuclei enlarge and pass to the base (Pl. 16, 2). Sinnott (1913) states that in the passage the two nuclei are accompanied by "most of the dense cytoplasm of the egg". This definitely does not occur in this material of *P. nivalis* as shown by Pl. 16, 2, which is typical of the many instances of this stage available. The upper denser cytoplasm is still commonly separated from the basal archegonial zone by a thinner vacuolated area. The two nuclei slip out from the upper cytoplasm, still clearly shown in the figure, and pass through this vacuolated area into the basal zone. This zone rapidly becomes denser and more clearly delimited, and in it the subsequent proembryo stages occur. The upper part gradually degenerates, though for some time the second male nucleus, as here, and other sterile nuclei may still be visible. Occasionally some of the sterile nuclei persist and, becoming enlarged and more tenuous, may migrate towards the embryo zone though never entering it. The second mitosis, again intranuclear, follows. The mitoses of Pl. 16, 3*a* and *b*, from two successive sections, are both rather high up and at the same level, but just as commonly one may be much deeper. Always however the second division took place in the basal zone. The four resulting nuclei (Pl. 16, 4) enlarge during a short resting period and then enter on the third mitosis outlined in Pl. 16, 5*a*, *b*, *c*, from successive sections.

At this stage a point needs noting. Later it will appear that in mature proembryos the number of cells is variable, a feature already recorded by the other workers. Thus Sinnott (1913) gives 7-10 as the suspensor number for *P. nivalis* and allied forms, Tahara (1941) speaks of 8-14 in the second tier of *P. macrophyllus*, and in all Buchholz's references there is a range, such as 10-14, for suspensor number in the species examined by him. For *P. nivalis* the explanation of the variability seems clear. Here, as also already recorded for *P. andinus* (Looby and Doyle 1944 *b*), embryonic nuclei, at different stages before primary wall formation, may be extruded from the basal cytoplasmic mass to lie free in the looser cytoplasm above. Here, later, they may perhaps even divide, but they play no part in the proper proembryo organization. Such extruded nuclei have been named *relict* nuclei by Looby and Doyle. If then this occurrence is authenticated in two species as widely separated as the stachycarpean *P. andinus* and the eupodocarpean *P. nivalis* it is a reasonable inference that such extrusion is a factor in the determination of the variability in mature proembryo numbers throughout the genus, even though the proembryo may develop along exactly the same plan and with the same number of free nuclear divisions.

In *P. nivalis*, then, following the third free mitosis there should be 8 free nuclei in the embryo cytoplasm as outlined diagrammatically in Pl. 16, 6. This type of course occurs, but Pl. 16, 7, on the other hand, shows an instance of one of the eight partly extruded, and in Pl. 16, 8 there is shown one already fully extruded and another nucleus which seems in the process of extrusion. An accurate estimation of the frequency of this extrusion can hardly be given, but of a dozen embryos at a stage to show 8 free nuclei three had a nucleus extruding or extruded and in two others extrusion was probable. Some of the others were still young, just after the third mitosis, and might well in nature have shown extrusion later. The phenomenon is thus reasonably frequent.

One applicable test would of course be the mitotic count at the fourth and last division. This division must be gone through very rapidly, as only two examples were found in the collections. One showed eight mitotic figures. Obviously there had been in this case no previous extrusion, but one of the mitoses was at the extreme edge of the cytoplasm and suggested the probable loss later of the two resulting nuclei. The other example was particularly clear, as the material was cut in transverse section and the simultaneous mitoses were all in late telophase. Four successive sections, including all the mitoses, are outlined in Pl. 16, 9 *a*, *b*, *c*, *d*. Here quite definitely there were only seven mitotic figures. The telophase chromosome clump in 9*a* was probably displaced a little in preparation, but the resulting nucleus would certainly have been at the extreme upper edge of the embryonic cytoplasm, and might well have been extruded later. Judging from the cell numbers at later stages, summarized in Tables 1 and 2, some further extrusion following this last division must also frequently occur, but it is not easy to produce visual evidence. The nuclei derived from the fourth division, 16 as a maximum but in many cases less, are at first somewhat irregularly crowded into

the small zone of dense basal cytoplasm, which stains almost as heavily as the nuclei themselves. It is thus difficult to be sure of the count at this stage. Also, by now, persistent sterile nuclei from the pollen tube might well have migrated to the neighbourhood of the embryonic cytoplasm, so that a distinction between extruded diploid nuclei and intruding haploid nuclei, if present in the area above the basal zone, was not usually possible. This final free stage is also passed through rapidly, so only a few instances are available of stages between the end of the fourth mitosis and primary wall formation. In none of them could the count of free nuclei in the embryonic cytoplasm be got up to 16, and in some it appeared to be as low as 10. However, although no quantitative estimation of relative extrusion frequency can yet be made, extrusion of potential embryonic nuclei from the embryonic cytoplasm undoubtedly occurs.

(*b*) *Mature proembryo.*

The free nuclei, whatever their number, then assume an orderly position in two tiers in preparation for membrane formation, the basal tier with only 1-3 of the nuclei (Pl. 16, 10 and 11). Pl. 16, 11 is from one of the sections of an embryo which had up to 12 nuclei in the main tier, and brings out the difficulty of a sure count, in longitudinal section, of these crowded nuclei when stain differentiation is poor. The two nuclei free in the non-embryonic cytoplasm above are probably two relict diploid nuclei. The condition, however, is again much clearer in transverse section, of which unfortunately only two instances were available at this stage. These showed nuclear tier numbers of 2 : 12 and 1 : 11 (Pl. 17, 12) giving in these two cases totals respectively of 14 and 12 nuclei incorporated into the embryo instead of the possible 16.

Delicate membranes now separate all the nuclei to produce the first walled stage of the proembryo of the form outlined in Pl. 17, 13 and 14. This is a definite and formal stage of the proembryo development, showing (*a*) a basal embryo tier of 1 to 3 cells, two in Pl. 17, 13 and one in Pl. 17, 14, and (*b*) what can be called the *open tier* as the cells are still unclosed by membranes towards the archegonium.

What may suitably be called the *internal division* now occurs, a fundamental phase in proembryo organization affecting *all* the nuclei. Divisions in the nuclei of the *open tier* are simultaneous, as are also divisions in those of the embryo tier, but division in the two tiers is not necessarily synchronous, though it may be. The original *open* tier divides with vertical spindles, on which membranes are normally laid down, to produce the *upper* tier, *U*, remaining open to the archegonium, and the closed middle *suspensor* tier, *S*, which will subsequently elongate. The divisions in the embryo cells lie at any angle, are without membranes, and produce the binucleate embryo cells, *E*, typical of podocarps.

The process is illustrated in Pl. 17, 15—18. Fig. 15 shows a case of synchronous division in the two tiers ; Fig. 16 one in which the divisions in two primary embryo cells are already complete, giving two binucleate *E* cells, while the open cell divisions are still in progress ; and Fig. 17 one in which the upper tier is already formed

before the mitoses in three embryo cells are over. Only three instances of mitoses in the *open* tier were met with of which two are illustrated, from successive serial sections, in Figs. 15 and 16. In both there are two embryo cells, but clearly many less than the requisite 14 in the upper mitoses if all of 16 nuclei were incorporated at the primary wall stage. The proembryo drawn in Fig. 16 can have had only 7, or perhaps at most 8, nuclei in the *open* tier when this was formed, giving only a total of 10 nuclei in all. In the case figured in Fig. 15 it is not so easy to be certain, but there cannot have been more than 10 in the earlier *open* tier giving with the basal two a total of 12. These numbers 10 and 12 must have resulted from four successive simultaneous divisions of the free embryonic nuclei. Wherever in the conifers the free nuclear stage of the early proembryo has been followed it has come from successive simultaneous divisions. Potential embryonic nuclei, such as those shown in Fig. 15*c* and 16*a*, must therefore have been lost, presumably by extrusion, in the course of development. The embryo of Pl. 17, 19, which is a transverse section through a recently formed *S* tier, shows 13 in this tier with which were associated two *E* cells, giving 15 in all, so that in other cases few or no nuclei may be lost.

Thus from the same basal plan and course of development there may arise considerable variation in the cell numbers in the mature proembryo produced by even one individual plant. But there is so produced in *P. nivalis*, the type proembryo outlined in Fig. 18, of three tiers—the embryo tier of few binucleate cells, *E* ; the suspensor tier, *S* ; and the upper tier *U*, a tier commonly ephemeral.

Before going further it seems well to summarize here data for cell numbers from actual available developing embryos, nearly 140 in all.

The numbers in the *E* tier could be definitely and easily determined, and there are here included not only those from the proembryos but also those from later stages, as the *E* cells can be clearly recognised for some time. These numbers worked out as follows :—

1 *E* cell, 45 ; 2 *E* cells, 80 ; 3 *E* cells, 10 ; and one doubtful *E* cell, 4.

It is not so easy to be definite about the numbers in the *S* and *U* tiers except in the limited number of embryos cut transversely, as numbers derived from longitudinal sections can be only approximate. These numbers are derived in practically all cases from proembryos before suspensor elongation. The suspensors grow very rapidly and, commonly, some cease to elongate at an early stage, or may not elongate at all, so that the number of suspensor tips in contact with developing embryos, though much more readily counted on a nuclear basis than at any other stage, may bear little relationship to the number in the *S* tier organized in the proembryo. Naturally too, in view of their origin, the open cells of the *U* tier equal those in the *S* tier, and can be recognized so to do in all transverse sections still young enough to retain them intact. So the *S* number in the following table is also the number for the primary *open* tier as well as for the *U* tier, and a mature proembryo with, say, 11 *U*, 11 *S*, and 2 *E* can be simply indicated as 11 : 2. The

E numbers again refer of course to the actual *cell* number, not to the *nucleus* number in the final stage which is double due to the development of the binucleate condition. Thus *E* numbers represent the number of embryo cells and nuclei at primary wall formation, and the sum of $S + E$ here given is a measure of the number of free nuclei incorporated at primary wall formation to give the open tier and primary embryo tiers.

TABLE 1.

Relative frequency of S : E in P. nivalis.

	A. All embryos				B. In transverse section only		
S No.	E1	E2	E3	Total	E1	E2	Total
7	1	0	0	1	1	0	1
8	0	4	0	4	0	2	2
9	4	5	0	9	2	2	4
10	2	7	0	9	0	2	2
11	7	8	1	16	3	1	4
12	2	5	1	8	0	1	1
13	0	6	4	10 ?	0	1	1
14	1	3	–	4 ?	0	0	0

TABLE 2.

Relative frequency of S + E totals in P. nivalis.

S + E =	8	9	10	11	12	13	14	15	16
All embryos	1	0	8	7	14	10	6	8	7
T.S. only	1	0	4	2	5	1	2	1	–

The numbers under S + E = 15 or 16 are probably too high, the 13 : 2 one in transverse section being the only case of these which can be relied on. These tables, however, do give at least some idea, even if only a very rough one, of the range of variation. Clearly the utilization of the full product of four mitoses, 16 nuclei, is relatively infrequent. Commonly there is a loss of at least one nucleus, most often of more than one. The authenticated case of a 13 : 2 proembryo in transverse section shows definitely that loss may occur in the course of, or after the completion of, the final division.

The isolated 7 : 1 proembryo may have a special significance. This was a clear example from transverse section showing quite definitely 7 *U* : 7 *S* : 1 *E* and no supernumerary nuclei which might be *relict* nuclei outside. This example

too is isolated, though this may be accidental, in so far as no proembryo was met showing the S + E total = 9. It is suggested that this may be a case in which one of the free mitoses was omitted, three mitoses yielding 8 nuclei, arranged 7 : 1 at the first wall formation. It is now certain, though the matter cannot here be discussed, that such dropping out of a mitosis, shortening the free nuclear stage, is a definite, even though quite an occasional phenomenon, in conifers.

Before leaving the proembryo it should be mentioned, merely as a matter of record, that one ovule showed two prothalli, each with a normal proembryo. The prothalli were unequal in size and asymetrically arranged, but clearly the archegonial apex of each must have been accessible to pollen tubes.

(c) *Suspensor growth and tetrad formation.*

The normal rapid extension of the suspensors, coiled and twisted in their upper length, now ensues pushing the embryo group *E* well down into the central cone of the gametophyte. This suspensor growth raises many problems. What factors determine that *S* cells elongate and *E* cells do not? How far is the penetration of the gametophyte mechanical or digestive? These and other problems have been long recognized and often discussed. There is however another problem which has not received much attention, viz. the actual origin of the extending walls of the suspensor cells. This problem arises rather acutely in *P. nivalis* because about the time of the *internal division* the wall of the rounded base of the archegonium becomes noticeably thickened. This has been noted and figured by Stiles (1912) for *P. macrophyllus* and by Sinnott (1913) for his New Zealand group. It is illustrated here, specifically for *P. nivalis*, in Pl. 16, 10, and Pl. 17, 13 and 16. Now at suspensor elongation the outer walls of the extending suspensors can hardly be these archegonial walls. What then is the relationship between the first proembryo membranes and the original archegonial wall? The relationship is still problematic, but it is here suggested provisionally that the proembryo is, as it were, carved out, *de novo*, from the embryonic cytoplasm, and that an outer membrane, quite separate from the archegonial wall, is secreted all round while the inner membranes are forming to separate the actual nuclei. This would give a better understanding of the later stages of development. As in the case of another membrane problem referred to later there are indications that the condition actually is as suggested, but the wall relations of archegonium and embryo must be left problematic till properly investigated. In the meantime in many of the proembryo figures a special membrane is in fact given to the actual embryo (cf. Pl. 17, 17 and 18) but this can only be taken as suggestive to call attention to the problem.

As the main group of the suspensors extend some commonly lag behind and may lose contact with the embryo. In other cases the original suspensor cells may never elongate and may be seen later as smallish cells at the top of the upper coiled zone. This dropping out may be slight or considerable so that in an extreme case from 12 original suspensor cells only 5 actively elongating units were later

found in contact with the developing embryo. This seems a common phenomenon in podocarps, at least in those with numerous suspensors, and has been known since Coker's (1902) original record. It was not possible, however, to gather any proper quantitative data in *P. nivalis* which might help in determining whether any regular features characterized the phenomenon.

At this stage an occasional suspensor may, though elongated, become separated from contact with the embryo (Pl. 17, 20), and the tips of these free suspensors may proliferate a little to give a small clump of embryo-like cells. This is quite a widespread phenomenon among *Podocarpus* species although probably less common in *P. nivalis* and its relatives than in many other species cited by Buchholz (1941). This feature will be briefly reconsidered later under polyembryony.

During the suspensor elongation the *E* group remains quiescent in the sense that the cells do not divide. They enter however into a peculiar resting stage essentially similar, except for the smaller number of cells concerned, to that described in *P. andinus* (Looby and Doyle 1944 *b*, Pl. 12, fig. 37). Nothing is known of the nature of this phase except that it shows itself by an intense cytoplasmic avidity for stain, which can only be removed by prolonged destaining, and by a tendency to gross plasmolysis with the formalin-acetic fixative used. The cells give every appearance of being dead and degenerating until suspensor elongation has slowed markedly or stopped, at which stage they show the first sign of returning to normal—the formation of the *cap*. The cytoplasm at the tip of each binucleate embryo cell, while still remaining dense, becomes separated by a thin zone (Pl. 18, 21) from the main bulk which retains the two nuclei. This small apical area is then cut off by the formation of a membrane across the thinner area (Pl. 18, 22). In this figure the main area containing the nuclei is indicated as still in the dense readily plasmolysed condition of the resting stage in contrast to the cell to the left of Fig. 21 which is shown as recovering. These two figures are illustrative of actual conditions, and show that, though a return to normal has been initiated as indicated by *cap* formation, visible cytoplasmic signs of change may appear slightly earlier or slightly later in different cases; but it is about this stage that cytoplasmic and nuclear recovery becomes evident (Pl. 18, 23). Stain avidity and the tendency to plasmolysis are less and the nuclear appearance alters. In the absence of proper cytological examination no exact account can be given of the nuclear change, but the nuclei, in the resting stage rather structureless and standing out but poorly from the cytoplasm, now seem, using terms very loosely indeed, more *formed*, with one or two distinct nucleoli around each of which is a definite clearer area. At this stage they closely resemble the corresponding nuclei recovering from rest in *P. andinus* (Looby and Doyle 1944 *b*, Pl. 13, fig. 43).

The membrane cutting off the *cap* raises a concrete problem to which reference must be made though a definite solution cannot yet be given. It is possible that in fact a new special wall membrane is formed enclosing the upper nuclear part of each *E* cell. This new membrane would be, in nature, in the closest contact with the original wall above and at the sides, but, below, would appear as the cap

membrane, which would thus be but the lower part of the new wall. Now, apart from the stain avidity of these embryo cells when resting, the cytoplasm, even after reawakening, is still dense during subsequent development, so that drastic destaining is still necessary for a reasonable demonstration of nuclei and chromosomes. As the stain mainly used, iron alum haemotoxylin, is a poor membrane stain the destaining thus necessary removed every tinge from all membranes. For this reason the existence of a new inner wall could not be definitely confirmed. The problem could be settled perhaps by suitably restaining the preparations, but as only a few good preparations of these early stages were available it was decided for the time being not to risk loss or damage to them in the hope that later search might provide more material which could be used to settle the point. In the meantime the inner membrane has been tentatively shown in certain of the figures, such as Pl. 18, 22 and 23, drawn from rather strongly plasmolysed cells in which appearances suggested that the plasmolysis had at places dragged the inner membrane away from the original outer wall.

The settling of this point is perhaps of more than mere cytological interest. If such a membrane exists the embryo cells might be considered to have developed, at this early stage, a certain independence from one another, an independence which becomes increasingly effective as development progresses. If, on the other hand, it is later shown that no such inner wall exists then the other problem arises concerning the contact and junction relations of the cap membrane with the existing walls of the embryo cells. Apart however from all these considerations the formation of a readily visible *cap* from each embryo cell is a fortunate circumstance for an investigator. As the embryo cells later divide to give various multicellular clumps the presence of the *cap* makes it quite easy to determine separately the products of division of each *E* cell.

With little delay after cap formation and reawakening to normal each *E* cell passes on to give a four-celled stage—the *embryo-tetrad* stage. This is a critical stage, probably common to all podocarps, which from the original binucleate cells produces uninucleate embryo cells in groups of four by the simultaneous division of the two nuclei in each *E* cell, (Pl. 18, 24). The independence, at least partial, of the *E* cells when there are two or three is perhaps shown by the fact that the *E* cells of any one embryo system do not keep in step. In no one of the six cases in which a double mitosis in *E* cells was shown were the *E* cells at the same stage. Thus in Fig. 24 the *E* cell to the right has already organized a tetrad while that on the left is still in the metaphase of the division. The actual manner of organization of the four *cells* from each *E* cell is still uncertain for *P. nivalis* as no stages are available between late telophase of the mitosis and an actual tetrad. But since, by late telophase, no traces of permanent spindles were apparent, nor traces of spindle membranes, it is probable that the behaviour, at least partly clarified in *P. andinus* by Looby and Doyle (1944 *b*), is here repeated.

Pl. 18, 25, a composite diagram from three sections, shows a typical result of the two tetrads from two *E* cells, though the distinctness of the tetrads has been

a little overemphasized. This figure shows some points of general interest. It shows how the caps help in determining the tetrad derived from each *E* cell. Without the caps, if the two original cells each tapered to an apex but kept in contact there might be some doubt, with the embryo group spread over several sections, whether it might not be in fact a more advanced stage derived from a single *E* cell. The figure also shows how each tetrad already gives the impression of a separate individual unit. It shows also on the right the four cells of the tetrad arranged tetrahedrally (cf. also Fig. 24) but a very different arrangement on the left. Clearly, too, in the cell group to the right one cell lies in an apparent apical position, a feature not shown on the left. It is probable that subsequent appearances are at least temporarily dependent on the accident of the lie of the spindles of the double mitosis and the relation of the resulting nuclei. Pl. 18, 26 *a* and *b*, from two sections, show a tetrad derived from a single *E* cell, as shown by the single cap, in which the spindles of the double division must have lain, the one horizontal the other vertical. Without the knowledge of a normal *embryo-tetrad* formation and without the *cap*, stages such as this might be open to a different interpretation. It might easily be claimed, for instance, that this showed the occurrence of two 2-celled units derived from two binucleate *E* cells by simple membrane formation in each cell between its two nuclei ; but such an interpretation would be incorrect.

(*d*) *Later development and polyembryony.*

An important feature of the continued development is the occurrence of a definite type of cleavage polyembryony not shown by the *E* 1 types but only by the *E* 2 and *E* 3 types.

In the *E* 1 types the cells of the single tetrad divide further advancing to a unified multicellular clump—a future potential embryo. Pl. 18, 27 shows, diagrammatically, an early stage in which the outline of the original cells of the tetrad is still visible. A division in the two lower cells would clearly produce a condition somewhat like a double tetrad from an *E* 2 type but the real nature is readily distinguished by the single cap. Pl. 18, 28 is from the central one of several sections of a more advanced stage. Whether the apparent apical cell functions as such is quite uncertain, but this is included as an example of the sort of appearance probably produced from a tetrahedral tetrad like that in Figs. 24 and 25. It is also uncertain whether there is any regular sequence in the earlier divisions. Otherwise the broad features of development seem normal. The embryo cells continue division and become relatively much smaller ; the apparent apical cell, in those cases in which it occurs at all, rapidly becomes embodied as it were in a general meristematic mass ; and the secondary suspensors, the embryonal tubes, form in succession by elongation of the proximal cells of the multicellular clump. Sufficient of these later stages are available to show that no splitting or cleavage occurs in these *E* 1 derivatives.

In each tetrad of the *E* 2 and *E* 3 types similar division occurs, but the resulting multicellular clumps remain visibly distinct as indicated by the diagrammatic outline in Pl. 18, 29 of a typical *E* 2 development. The separate nature and independence at this stage of each of the *E* cell derivatives is further shown by the fact that one of the tetrads from, say, an *E* 2 type may lag markedly behind the other. This is seen in outline in Pl. 18, 30, in which the left segment is still at the 4-celled stage, and in Pl. 18, 31, from an *E* 3 type, in which one of the original binucleate *E* cells, that on the right, never even advanced to the double mitosis. The other segment, developing well, is an outstanding example of the apical cell appearance already considered. The third segment in this system lay in other sections and is not drawn, but it too was in active development. Early lagging of this sort is of course not typical; the typical and common condition is that of Fig. 29, nor of course was the apical cell appearance as common as the figures reproduced might suggest.

Each cell clump then, each derived from a tetrad which itself came from an original binucleate *E* cell, can develop into a separate embryo as shown by Pl. 18, 32 to 35,—Figs. 32 and 35 from embryos dissected out whole, Figs. 33 and 34 from sectioned material. Clearly the potential embryos ultimately separate from one another as really independent units due to the formation and extension of the secondary suspensor system produced by each.

These four figures are simply illustrative of commonly occurring conditions. Each of them shows one embryo system, the embryo clumps in each case being definitely connected to one suspensor system which, at this level, may show many or only a few suspensors. Fig. 32 shows three multicellular clumps still closely connected with a normal suspensor group. Fig. 33 shows an example with few surviving suspensors and with two original cell groups, one of which has not developed, while the other has a strong group of embryonal tubes which have already pushed up the primary suspensors in contact with them. Fig. 34 shows two well-developed clumps in one of which the embryonal tubes have been more active than in the other and have also begun to push away their associated primary suspensors. In Fig. 35 there are again three embryos, the one with an early development of embryonal tubes having become separated in dissection. The embryonal tubes elongate relatively rapidly, completely separating the embryo clumps, whose cells are in active division.

Development was not followed in detail any further. Though a number of scattered later phases are available they are omitted from this account as they do not suffice to give a clear connected story. As usual only one of the embryos develops to maturity, and its development is slow. By late November it shows barely the first organization of a plerome centre and a short calyptroperiblem; not till well into the succeeding year is the mature form assumed. It is not proposed here to deal with this aspect of later embryogeny except to comment that Brownlie's (1953) Fig. 16 of a "well-advanced" embryo seems an inadequate representation of the very varied structure shown in the present material. However, the organization

of the mature embryo of the seed from the primitive multicellular clumps raises so many complex problems of form, development, and cellular behaviour, that it must be a specialist study. The recent works for example of Schopf (1943) on *Larix*, Allen (1946) on *Pseudotsuga*, Holloway (1937) on *Phyllocladus*, and Brownlie (1953) on New Zealand species of *Podocarpus*, in addition to those of the older workers such as Schacht and Strasburger, illustrate only too clearly the complexity of the development, and are also a reminder that a comprehensive comparative survey of such organization in conifers has still to be made.

(*d*) *Anomalies.* The course of development outlined above for *P. nivalis* seems, allowing for the variation range in the number of free nuclei used, to be quite stereotyped. Only two anomalous cases, both of the same nature, were met with. In these two instances the only embryo structure to be found in the ovule, well down in approximately the normal position, was a small group of cells attached to the tip of a wide single suspensor (Pl. 18, 36). These must be considered degenerate anomalies. In normal development the main bulk of the upper entangled lengths of the coiled suspensors can be seen quite definitely and distinctly, even up to stages such as those shown in Figs. 32—35, and are then still so compact and firm that their complete collapse and disappearance could only occur much later. In these two anomalous ovules however the whole suspensor group had broken down into a degenerate almost structureless mass. The single suspensor surviving is quite different in nuclear form and position, as well as in its intenser vacuolation, from normal suspensors as shown in any of the earlier figures (cf. Figs. 21, 24). The 'embryonic' cells too, of which perhaps another two or three are to be found in the succeeding section, differ from true actively developing embryonic cells in their much more delicate cytoplasm with many vacuoles, and in their rather fragile nuclei. There is no *cap*.

These, the only two anomalous cases found, claim this mention as they probably bear on Coker's (1902) suggestions on embryo splitting.

C. Comparison with other *Eupodocarpus* Species.

The early embryogeny of *P. nivalis*, as here outlined, seems to be quite representative of development in the *Eupodocarpus* section. It shows a definite plan, but, arising from the extrusion of nuclei during the free nuclear stage, shows also a wide variation in the number of cells found in mature proembryos. Differences recorded by other workers fall within that variation range.

Division of the fusion nucleus *in situ* and the passage of the resulting two nuclei to the base, which had been recorded though not figured by Sinnott (1913) for his New Zealand species, are confirmed. Coker (1902) did not find this stage in the species investigated by him, and had inclined to the view that the fusion nucleus might first have migrated to the archegonium base and there divided, a statement categorically repeated by Stiles (1912) for *P. macrophyllus*. As Stiles gives no

evidence, as he most probably did not actually see the first stage but simply followed Coker, and as Coker in fact left the matter very much in doubt, this confirmation of Sinnott's statement could be fully accepted were it not for Tahara's (1941) statement. He claimed for *P. macrophyllus* two sporophytic divisions *in situ* giving four nuclei passing to the base. His evidence however is quite inconclusive. He figures neither the first nor the second mitosis, and illustrates the supposed 4-nucleate stage in the upper archegonium (his Pl. 2, Fig. 3) merely by a small mass of cytoplasm in which lie *two* nuclei only. The location of the other two nuclei claimed is not shown, nor are the relations of the supposed four to the whole archegonium indicated in any way. Coker's figure (his Fig. 32) on the contrary is unmistakably that of a 2-nucleate stage at the archegonium base, and shows that the fusion nucleus by that time had only undergone *one* division. As Coker's plant was a variety of *P. macrophyllus*, as there is no record in any other podocarp of the fusion nucleus passing to the base *before* division, and as Tahara probably saw some mitosis *in situ* above, it seems a reasonable inference that Sinnott's record, here confirmed in *P. nivalis*, holds for *P. macrophyllus*. No other evidence being available, it is here taken, pending data to the contrary, that division of the fusion nucleus *in situ*, the resulting two nuclei passing to the base, is characteristic of *Eupodocarpus* species.

Three free mitoses follow, giving a possible maximum of 16 free nuclei—on this point there is common agreement. The extrusion during the free nuclear phase of *relict* nuclei, necessary to give the wide variation in final nuclear number incorporated in the proembryo, does not seem however to have been realized by any of the authors cited, though such relict nuclei were probably visible enough in some of their preparations. Thus, as examples, the nucleus shown just above a mature proembryo of *P. nivalis* in Sinnott's photograph (his Pl. 7, Fig. 31) is almost certainly such a relict nucleus, and the two free nuclei above in Tahara's figure of the *internal division* in *P. macrophyllus* (his Pl. 2, Fig. 7) are most probably relict nuclei also.

The existence of the two clear phases in the organization of the walled proembryo, i.e. the first phase of wall formation giving the *open* tier and the primary embryo tier, followed by the phase of the *internal division* giving the upper *U* tier, the middle suspensor tier *S*, and the embryo tier *E* of binucleate cells, does not seem to have been properly appreciated either, except perhaps by Tahara (1941). Sinnott (1913), followed by Brownlie (1953), unaware of the *internal division*, considered that the free nuclei were directly organised into three tiers. Thinking in terms of 16 free nuclei the 7—9 recorded suspensors and the single recorded binucleate cell would leave 4—7 ephemeral nuclei in the upper tier, a tier to which he gave no attention. Had Sinnott encountered proembryos with higher *S* numbers he must have realized the difficulty already faced by Coker (1902) seven years earlier. Coker had recorded a proembryo with 14 *S* cells and one binucleate *E* cell as well as an upper tier of open cells coinciding in position with the cells of the *S* group. Even though the number of *S* cells may not have been accurately 14 it was certainly high, and

the whole clearly could never have come from 16 free nuclei. It was unfortunate that Sinnott should have met a proembryo of *P. nivalis* in the lower range, because *his* ideas of development, rather than Coker's, influenced Buchholz as it currently influenced Brownlie. In fact, Coker's original suggestion first made more than 50 years ago was thus correct and has been here formally confirmed—the origin of the *upper* tier from the primary *open* tier in the course of an *internal division.*

Tahara (1941) however, although his account of development in *P. nagi* is accurate and precise, left the position in his material of *P. macrophyllus* unsatisfactory and unconvincing, and made no attempt at comparisons between them, although both embryogenies are recorded in the same brief paper. He states that at first wall formation two *or* three tiers may be formed. If two tiers they are a single terminal binucleate cell and a second tier of 14 cells. Although in the same paper he figures divisions in the primary embryo cells in *P. nagi* to give the binucleate condition, no reference is made of such a division in *P. macrophyllus*, the impression being given that the two nuclei in the terminal cell are the other two of the original 16 free nuclei. However he further states that the 14 cells of the second tier, open above, *may* divide by vertical spindles to give an upper tier and a true suspensor tier, a stage which is figured. The nature of the internal *division* affecting *all* the nuclei is not made clear, but at least the existence of a division affecting the original second tier is recorded. He further states however that such a division may not occur but that the primary second tier may be closed above thus forming a proembryo of two tiers only—a suspensor tier and the single embryo cell. No information is given concerning the relative frequency of these two conditions or the absolute frequency of the second, and so, even if confirmed, the second condition may be merely a rare anomaly. Its occurrence, as in any way a typical or common feature, is altogether unlikely. Wherever in podocarps information is available on proembryo stages an upper tier is recorded, though the tier may be quite ephemeral. It is much more probable that any instance giving the appearance drawn by him in his Pl. 2, Fig. 9, a figure claimed to illustrate a mature proembryo in which an upper tier was never formed, is merely an example of particularly early degeneracy of the upper tier.

Tahara (1941) records further that the suspensor tier number may vary from 8 to 14 and then states that, if " less than 14 nuclei " go to make the primary second tier, then three tiers are formed directly from the primary nuclei without any internal division. This statement is very ambiguous and he gives no evidence in support of it except reference to his Pl. 2, Fig. 8. He does not indicate what happens if there are say 12 nuclei in the primary tier, nor does he give any information concerning the relative number of nuclei in the final upper and suspensor tiers in these cases. Clearly with 12 nuclei in the second tier and two in the embryo tier there could, from 16 primary nuclei, be at most only two in the upper tier in the absence of an internal division. It would appear that, like Sinnott, he was thinking in terms of lower suspensor numbers, such as 8, and the utilization of all 16 primary nuclei, which could be arranged 2 : 8 : 6 if neither internal division nor extrusion

were recognised. Recognition of the fact of extrusion, as demonstrated for *P. nivalis*, resolves all such difficulties and permits a similar plan of development to produce proembryos with greatly varying suspensor numbers while still retaining the internal division. It is, incidentally, a very probable inference from a survey of all conifers, that an *internal division* is a fundamental feature of conifer embryogeny, an aspect which it is hoped to elaborate on another occasion. Its omission from certain proembryos of *P. macrophyllus* cannot, on the evidence, be accepted.

Examination of the numbers in the proembryo tiers of eupodocarps recorded so far in the Literature shows that they all fall within the range of variation here given for *P. nivalis*. The records available are almost all for the *S* and *E* tiers the ephemeral *U* tier being commonly disregarded, and are as follows :—

TABLE 3. *S : E numbers in eupodocarps*

	S		E	
P. nivalis	8 — 14	:	1 — 3 (mostly 2).	Here cited
P. macrophyllus *v.* maki.	14	:	1	Coker (1902)
P. macrophyllus *v.* maki.	about 14	:	1 (rarely 2).	Buchholz (1941)
P. macrophyllus.	8 — 14	:	1 (once 2).	Tahara (1941)
P. totara.	7 — 10	:	1 (rarely 2)	Sinnott (1913)
P. totara *v.* Hallii.				Buchholz (1941)
P. nivalis.				Brownlie (1953)
P. urbanii.	12 — 14	:	2 — 3	Buchholz (1941)
P. glomeratus.	about 12	:	3	,,
P. coriaceus.	11 — 12	:	2 — 3	,,
P. purdeanus.	11 — 12	:	?	,,
P. matudai (?).	11 — 12	:	2	,,

The *S* numbers for the New Zealand species, originally given by Sinnott, and apparently simply accepted by Buchholz and Brownlie, appeared exceptionally low before this study of *P. nivalis* was made. Buchholz commented on this, and seemed to think the point of taxonomic importance, but in fact there is nothing exceptional in *P. nivalis* nor, by inference, in the other species. The lower record of Sinnott was most probably simply due to the accident that the few proembryos he handled happened to be in the lower part of the normal range. Also, as these records in Table 3 are almost certainly all from observations on a limited, often a very limited, number of embryos, it is again probable, as suggested by the 8—14 range for Tahara's *P. macrophyllus* material, that they all would also show as wide a range as *P. nivalis* if equal numbers of embryos were examined. Similarly, since Sinnott's record of *E* number in *P. nivalis*, 1 (rarely 2) should be in fact 1—3 (mostly 2), too much reliance cannot be placed on the apparent regular occurrence of only one *E* cell in *P. macrophyllus*, though it is quite possible among the large group of eupodocarps that, in certain species or groups of species, the tendency

towards a small number of *E* cells shown by *Eupodocarpus*, in contrast to other sections, may have advanced to its limit.

The lagging in development, or the non-development, of suspensor cells seems common and widespread in the section, but there is no other record of the peculiar resting phase of the *E* cells during suspensor elongation. Since it has also been reported for *P. andinus* (Looby and Doyle, 1944 *b*) it is suggested that it is at least a generic character to be expected in all species of *Podocarpus*. The recent report by Elliot (1950) of a similar occurrence in *Pherosphaera* makes it possible that it is, like the binucleate condition of the *E* cells, a constant feature of all podocarps.

The manner of formation of what has here been simply called the *cap* has not previously been noted, but this *cap* is quite probably a feature of development in *Eupodocarpus*. Brownlie (1953) refers to the terminal binucleate cell being protected by a " thickened cellulose cap " but gives no clue to its origin. In his Figs. 1—8, which include drawings of young *E* cells and of multicellular embryo groups, this " cellulose cap " is shown as a small projection which represents, without question, the *cap* structure whose origin has been shown earlier in this paper. The *cap* too is clearly not limited to the New Zealand group of species, as two of Buchholz's figures (1941, Figs. 26, 27) of a young multicellular embryo stage in his material of *P. macrophyllus* also show, but without comment, a small projection at the tip of the embryo which is exactly the appearance given by the *cap* at this late stage. There is of course no evidence that the cap function is 'protective', especially as it does not develop until suspensor growth has slowed markedly or stopped.

The divisions in the binucleate *E* cells which initiate, through the *embryo-tetrad* stage, the uniucleate condition of the later embryo cells were first appreciated by Buchholz (1941). Coker (1902) had stated that the first division in the *E* cell, single as reported by him, was by a longitudinal wall, but he did not consider the relations of the wall to the nuclei. Assuming, as he did, only one *E* cell he illustrates, in his Fig. 40, this first 2-celled derivative. But this figure is in fact one of a young embryo with 2 *E* cells as shown by the binucleate condition of the cell on the right. The second longitudinal division was merely inferred by Coker on no evidence, although it is possible that he may have seen an early tetrad. Sinnott (1913), also ignoring the problem of nuclear change, states that the single embryo cell, recorded by him for the New Zealand species investigated, first divided by a longitudinal wall and then by transverse ones. Again no evidence is given for the statement, but he too may have seen an early tetrad. Buchholz (1941) however, produced the first exact evidence of the actual behaviour. In his material of *P. totara* a single *E* cell occurred almost exclusively but in one of the few systems with 2 *E* cells he was fortunate in finding one of the cells with its nuclei in simultaneous division and the other in a tetrad condition (cf. his excellent Fig. 33). Recognizing further the occurrence in other species of uninucleate cells in groups of four at the tip of the elongated suspensors (cf. his Fig. 3 for *P. urbanii* or Fig. 23 for *P. macrophyllus*) he drew the inference that what is here called the *embryo-tetrad* stage was a characteristic feature not only of *Eubodocarpus* but in all podocarps.

"It is probable that in all podocarps the binucleate cells directly form similar 4-celled groups". This was a broad inference from slender evidence, but it is unquestionably sound and will be considered more fully later in the section on embryogeny in podocarps in general. For reasons there outlined it was necessary to confirm beyond doubt Buchholz's claim for the occurrence of an *embryo-tetrad* stage in eupodocarps. This has now been done for *P. nivalis*.

It has also been shown for *P. nivalis*, where more than one *E* cell is present, that each *E* cell may develop independently through the tetrad stage to a small multicellular clump—a potential embryo. These are separated into distinct growing units by the elongation, in each, of its proximal cells as embryonal tubes or secondary suspensors. The origin of supernumerary embryos, the nature of the polyembryonic cleavage, has here been followed in exact sequence for the first time in any species of *Podocarpus*.

This type of cleavage must be common in *Eupodocarpus* as appears from Buchholz's (1941) paper on embryogeny in the Family. The part of that paper concerned with *Eupodocarpus* definitely records cleavage in *P. urbanii* and *P. glomeratus*, and, considered in the light of the close sequence here followed out in *P. nivalis*, re-examination of Buchholz's figures and script shows that the sequence in these must have been essentially similar. Thus in *P. urbanii* his Fig. 7 shows embryos with 2 or 3 *E* cells still binucleate, Fig. 8 shows 3 derived tetrads, Fig. 18 shows 3 multicellular clumps still in contact with the original suspensors, Fig. 13 another instance with 2 such clumps, and Figs. 9 and 10 indicate the separation of these clumps by the elongation of their respective embryonal tubes. From herbarium material of *P. glomeratus* only one good embryo system was dissected, but it is described as showing 3 compact embryonic clumps arranged essentially as in his Fig. 18 of *P. urbanii*. Although he records the occasional occurrence of 2 *E* cells in *P. totara* and *P. macrophyllus* he met no example of cleavage, presumably because material was scanty, but he is quite definite that no cleavage occurs with only one *E* cell in the early stage. He seems to suggest that cleavage might be expected to occur in those cases in which two *E* cells were present.

It seems then that the interest of the account of actual embryo cleavage given here for *P. nivalis* is that it essentially clarifies and confirms conceptions of cleavage which Buchholz apparently had, but which he had not succinctly formulated. He, however, goes farther since he includes under cleavage polyembryony any occurrence of proliferation, even if slight, at the tips of suspensors which have become separated from the others and from contact with the embryo cells proper. This, as well as his comments on 'rosette' embryos, will be considered later in the section on embryogeny and polyembryogeny in the podocarps as a whole, so that here it must suffice to suggest that only confusion can arise if true embryo splitting and free suspensor proliferation are considered but different aspects of the one phenomenon. It is confusing the issue to attribute cleavage polyembryony, unqualified, to two species A and B if, on reference, it appears that A had only one *E* cell and some

S proliferation while B had three separating *E* cells and no proliferation. Apart from such considerations proliferation of free suspensor tips must be widespread in eupodocarps if only as an occasional phenomenon. In some cases however it may go to extremes, and the whole group of suspensors may separate from one another and from the *E* group to form a tangled mass of single tubes with proliferated tips, such as that illustrated by Buchholz in Fig. 16 of his account of *P. urbanii* and recorded as occurring in about ten per cent of the ovules examined. Such occurrences can only be regarded as cases of complete breakdown of normal embryonic organization, but they bear directly on Coker's (1902) account of " budding " or " splitting " in his material.

According to Coker his eupodocarp material had only one *E* cell, which first divided into four by two longitudinal walls. It will be remembered that the figure which he gave to illustrate the first longitudinal division really shows a young stage with 2 *E* cells, and also that the occurrence of a second longitudinal division was merely inferred from the nature of the subsequent " splitting " which he records. He recognized that most frequently no splitting occurred, the single *E* cell developing directly into a single embryo, but he considered, in those cases in which " splitting " occurred, that the four supposed derivatives from the *E* cell could separate each taking with it a single freed suspensor tube at the tip of which it developed. This supposed splitting of the embryo cell, the product of one archegonium, into four is, he says, " frequent " but no indication is given as to the number of cases in which it was actually met. His scanty evidence is summarized in his Figs. 43 and 44. The latter figure simply shows the lower part of *three* separate single suspensor tubes each with a small group of cells at the tip. The expected fourth is not shown, nor is its absence commented on, nor is there any evidence even that the three drawn came from one archegonium. The former figure, Fig. 43, shows a similar three with, above them but much higher in the prothallus, a well-formed multicellular clump attached to what appear to be the tips of a unified group of normal suspensors. The presence of this clump, which must be an embryo, is not explained. His reference to this, that " the three lower embryos have come with one other from a single archegonium ; in this case the suspensors not having separated " is ambiguous. Apart again from the absence of evidence to show that this system did in fact come from one archegonium there appears to be the underlying suggestion that, of the 4 cells into which the original *E* cell is supposed to have divided, three separated with one suspensor each and the fourth remained in contact with the rest of the suspensors.

This of course cannot be fitted in with the known behaviour of the *E* cells in *P. nivalis* or normal *P. urbanii*. Buchholz (1941) however has pointed out that these appearances do fit in with the known occurrence of the proliferation of free suspensors, a suggestion which explains the situation and which is here fully accepted. It has been shown that proliferation of the tips of free suspensors, though not common, may occur in *P. nivalis*, and that, as a rare anomaly, degeneration of almost the entire suspensor system may take place leaving, in the few cases

noted, but one suspensor tube carrying a few cells which probably would never develop into an embryo. Proliferation may be much commoner in other species, such as *P. urbanii* and *P. macrophyllus* (Buchholz, 1941, Tahara 1941), and in these also considerable disorganization of the suspensor system may occur. Coker's Fig. 44 must be one such in which the central embryonic organization has broken down leaving temporarily but three free suspensors with proliferating tips. The other figure, Fig. 43, may show either a similar development from one embryo system with a more normal embryo from another fertilization or one normal system with three proliferating suspensors, in both cases development of the more normal embryo having been retarded. Such retardation may have been frequent enough in Coker's material, as he draws, Fig. 41, a case of a well-developed embryo mass attached to a group of suspensors which have undergone but little elongation, a condition quite abnormal for podocarps. Coker's embryo " splitting " must have been simply a rather abnormal condition of suspensor proliferation, the apparent frequency of the anomaly being related perhaps to the garden origin of his material of *P. macrophyllus*. As neither Stiles (1912), Buchholz (1941), nor Tahara (1941) met true embryo cleavage in their material of this species, although Buchholz and Tahara record that free suspensor proliferation is common, true embryo cleavage must be rare in it, possibly because the occurrence of more than one *E* cell is not common.

Sinnott (1913) added nothing on the problem of cleavage in his work on New Zealand eupodocarps. He merely stated, giving no detail and obviously influenced by the earlier ideas of Coker, that " instances of budding or of single suspensors giving embryos were rarely observed ".

Brownlie (1953) too adds nothing to our knowledge of cleavage in eupodocarps in spite of a familiarity with Buchholz's (1941) paper. It was earlier mentioned that, apparently without personal investigation, he seemed to accept Sinnott's (1913) concept of proembryo formation and with it the occurrence of only one *E* cell except as a very rare condition. Since there can be no cleavage with only one *E* cell it is perhaps not remarkable that in the material of the four species investigated he found only two reputed examples of cleavage, illustrated in his Figs. 21 and 22. The latter figure almost certainly represents an anomalous condition somewhat like that described by Coker, though Fig. 21 probably does represent two embryo systems with, in each, two *E* cells developing separately. The actual species in which these occurred is not stated, nor does either figure give any clear indication of the details of origin of the structures concerned. In view of conditions in *P. nivalis* the apparent rarity of cleavage in Brownlie's New Zealand material would seem to be due to the examination of too little material from too many forms. He adds, however, *P. acutifolius* by name as a species in which proliferating free suspensors occasionally occur as they do in *P. nivalis*, and suggests, probably quite correctly, that such a phenomenon may be, in fact, rarer in these New Zealand types than in forms like *P. urbanii*.

From this discussion, which has endeavoured to include all pertinent detail, it may be concluded, pending much wider investigation among the many species of *Eupodocarpus*, that the course of early embryogeny here outlined for *P. nivalis* may be taken as representative of at least typical members of the section.

Summary.

Embryogeny from fertilization is described for *P. nivalis*. The fusion nucleus divides once *in situ*, the two nuclei passing to the base, where three further simultaneous free mitoses occur—the *free nuclear* stage. During this stage a variable number of embryonic nuclei, the *relict* nuclei, may be extruded. At primary membrane formation the remaining embryonic nuclei, 10 to 16 in number, are arranged in two tiers—a lower primary embryo tier of 1 to 3, most often 2, cells, and an *open* tier, not walled above, of 8 to 14. An *internal division* then affects *all* nuclei. Vertical spindles in the *open* tier give an *upper* tier *U*, usually open above, and the *suspensor* tier *S*. Divisions in the primary embryo tier give the typical podocarpean binucleate embryo cells, *E*. This is the *proembryo* showing, *U* 8—14, *S* 8—14, *E* 1—3. During *S* elongation the *E* cells enter into a resting phase. Later, returning to normal, each *E* cell cuts off part of its apical cytoplasm to form the *cap*. Simultaneous divisions of the two nuclei in each *E* cell, followed by membrane formation, produce from each *E* cell an *embryo-tetrad*. Each tetrad continues division independently of the others so that 1, 2 or 3 multicellular clumps develop—the potential embryos. If 2 or 3 in number the potential embryos are separated by the development of embryonal tubes from the proximal zone of each—*cleavage polyembryony*. With only one *E* cell there is no cleavage. Slight proliferation of the tips of free suspensors may occur. It is possible that, very rarely, one of the free mitoses may be dropped giving arrangements such as 7 : 1 at primary membrane formation.

A critical survey of all available data in eupodocarp embryogeny shows that the sequence of development in *P. nivalis* is typical of the section though the actual number of cells in the proembryo tiers may vary.

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EXPLANATION OF PLATES.

Pl. 16.—1. First sporophytic mitosis. x 280. July 15. 2. Two nuclei in archegonium below; second male nucleus and one other sterile nucleus above. x 280. July 23. 3 *a*, *b*. Second mitosis. x 375. July 21. 4. Four-nucleate stage. x 375. July 27. 5 *a*, *b*, *c*. Third mitosis complete; three consecutive sections. x 375. July 22. 6. Eight-nucleate stage. x 280. July 22. 7, 8. Extrusion of relict nuclei at 8-nucleate stage. x 375. July 25. 9 *a-d*. Fourth mitosis; consecutive sections all divisions included. x 375. July 23. 10. Nuclei arranged for membrane formation; *ac* thickening of archegonial wall; at least one relict nucleus. x 375. July 23. 11. Another of same with two relict nuclei showing. x 375. July 23.

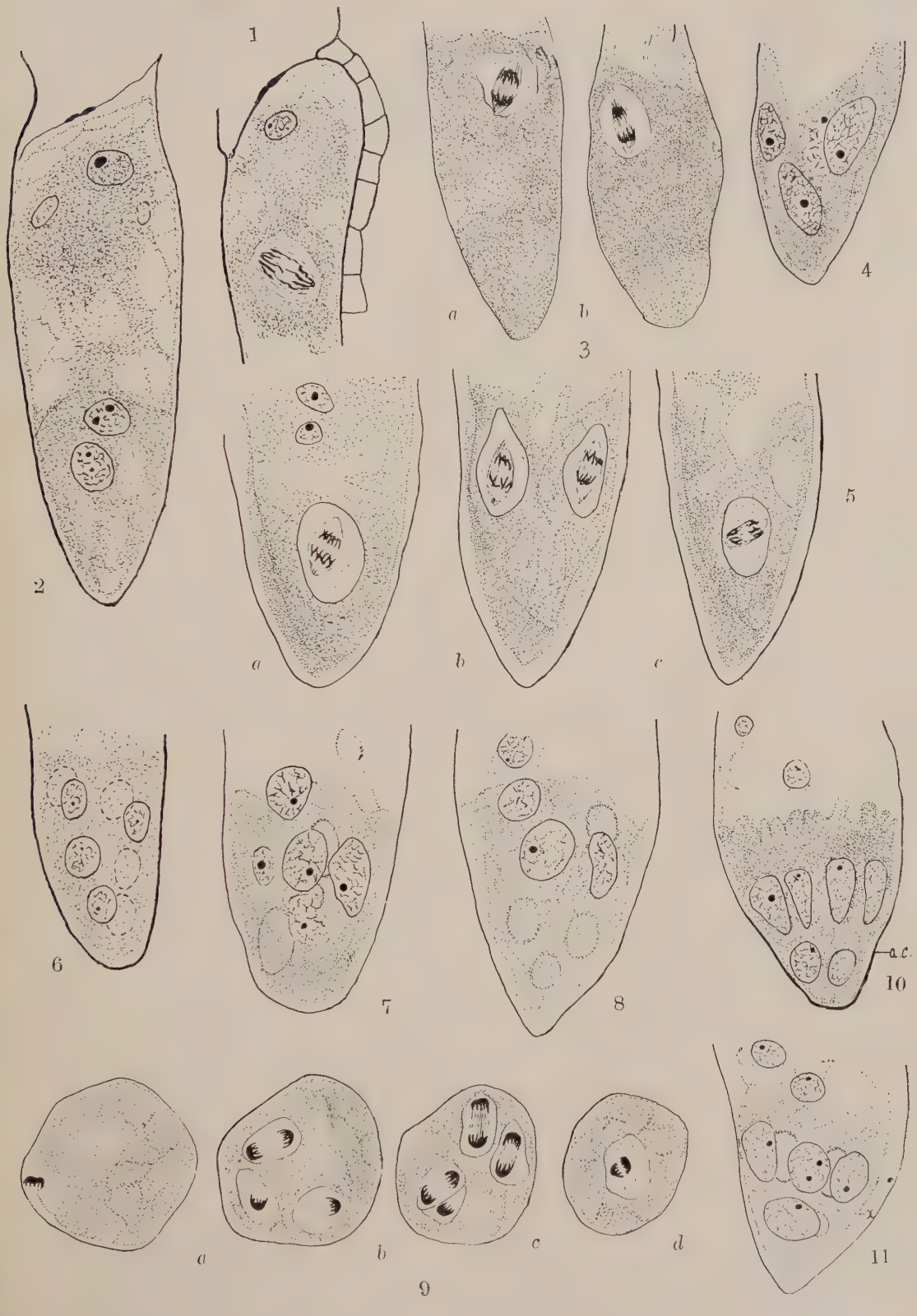
Pl. 17.—12. T.S. upper tier of arranged nuclei before membrane formation showing only 11 nuclei. x 375. July 21. 13. Early membrane formation giving *open* tier and embryo tier with, in this case, two primary embryo cells. x 375. July 27. 14. The same with one embryo cell. x 375. July 27. 15 *a-f*. Series of consecutive sections showing *internal division* affecting *all* nuclei; all divisions in this case simultaneous; two embryo cells; in *c* two *relict* nuclei. x 375. July 27. 16 *a-d*. Internal division already complete in the two binucleate *E* cells; divisions in *open* tier simultaneous; thickening of archegonial base *ac* indicated; in *a* one relict nucleus. x 375. July 25. 17 *a*, *b*. *Upper* tier *U* and *suspensor* tier *S* formed from divisions as in figs. 15, 16; divisions in the 3 embryo cells still in progress. x 375. July 24. 18. Proembryo formed; *U* tier, *S* tier and binucleate *E* cells, in this case only one. x 375. July 24. 19. T. suspensor tier with 13 cells; (c.f. fig. 12). x 375. Aug. 2. 20. Later stage of *S* elongation; *E* cells at tip still binucleate; one free proliferating suspensor, *fps*. x 75. Aug. 20.

(Note:—In figs. 17 and 18 the *problematic* distinctness of embryonic membranes and archegonial wall is indicated).

Pl. 18.—21. Early stage of *cap*, *c*, formation in resting *E* cells. x 400. Aug. 20. 22. Cap, *c*, cut off; *E* cells still in resting stage. x 400. Aug. 18. 23. *E* cells returning to normal; *c*, caps. x 400. Aug. 20. 24. *Embryo-tetrad* formation in two *E* cells, each with cap, *c*; simultaneous division of the two nuclei in the *E* cell to left resulting in tetrad of uninucleate cells as on right. x 400. Aug. 20. 25. Two *embryo-tetrads* from two *E* cells as shown by caps, *c*. x 400. Aug. 18. 26 *a*, *b*. Single tetrad from one *E* cell, one cap only; two successive sections. x 400. Aug. 18. 27. Single tetrad, one cap, initiating post-tetrad divisions. x 400. Aug. 18.

28. Central section of more advanced multicellular derivative of single tetrad ; apparent apical cell. x 400. Aug. 20. 29. Typical multicellular derivatives of two tetrads, two caps. x 400. Aug. 16. 30. Example of unequal development of two tetrad derivatives, one on left still at tetrad stage. x 400. Aug. 24. 31. Extreme case of unequal development ; *E* cell on right still at binucleate stage. x 400. Aug. 18. 32. Three potential embryo clumps from three *E* cells ; caps still visible ; from dissection. x 150. Aug. 24. 33, 34. Separation of potential embryos by elongation of embryonal tubes, *et* ; embryo on left in fig. 34 lagging. x 150. Sept. 1. 35. Three potential embryos from dissected system ; embryo with early embryonal tubes separated in dissection. x 150. Aug. 28. 36. Anomalous embryo cells, no cap present, attached to single suspensor (see text). x 400. Aug. 24.

(Note :—In figs. 22, 23, 27 and 30 the *problematic* separate inner membrane cutting off the functional part of the *E* cells is suggested ; see text).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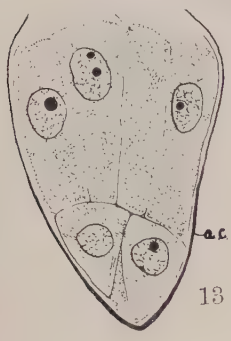

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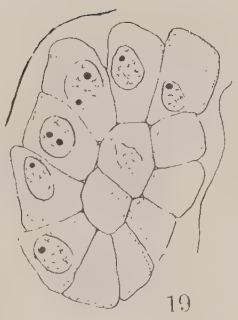

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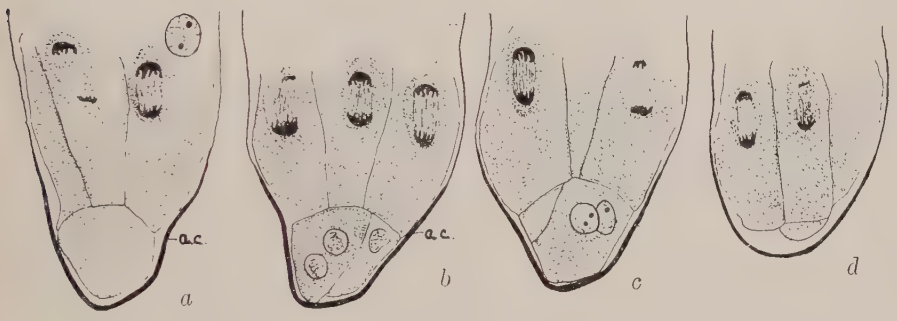

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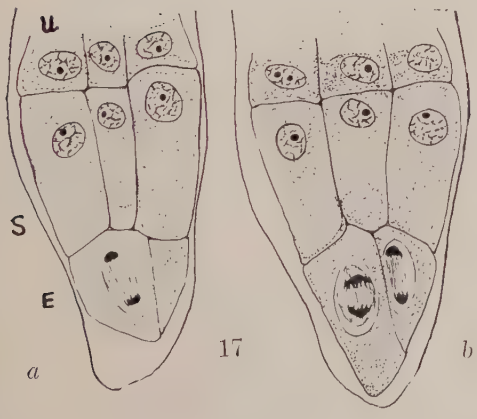

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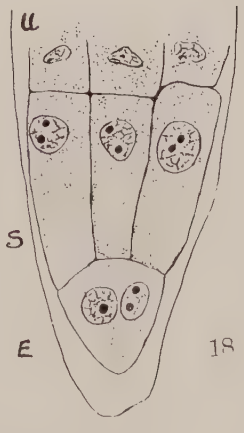

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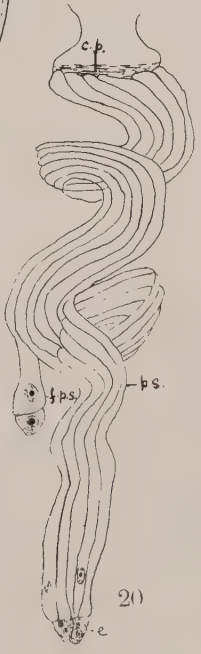

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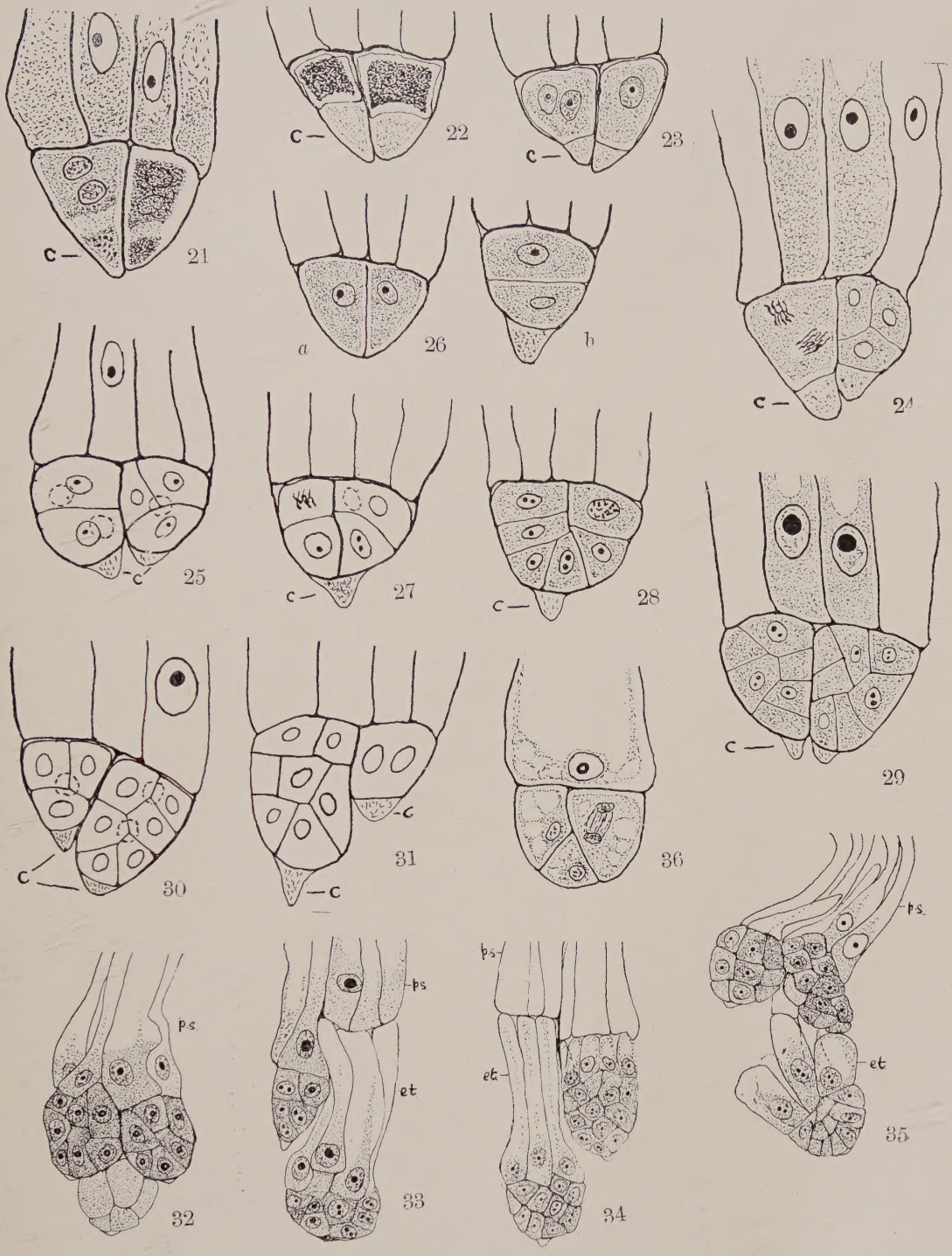
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